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Letter to the Editor regarding Qui et al: "*Cutibacterium acnes* and the shoulder microbiome"

To the Editor:

We share the interest of Qui et al[10] in understanding the potential existence of endogenous *Cutibacterium* (formerly *Propionibacterium*) *acnes* within the glenohumeral joint and agree that advances in our understanding of the surgical-site microbiome may yield new avenues for prevention of surgical infections. In their recent article characterizing the shoulder microbiome, Qui et al used 16S bacterial ribosomal RNA (rRNA) amplicon sequencing to quantify bacterial species present in skin, subcutaneous fat, and rotator cuff tissues. The reported absence of *C acnes* in the skin and subcutaneous fat samples is an unexpected finding and contrasts with several prior studies in which *C acnes* has consistently been cultured from shoulder samples following standard skin antisepsis measures.[2,6,7]

A likely explanation for this apparent discrepancy is that the polymerase chain reaction (PCR) primers selected for the study are unable to detect most strains of *C acnes*. As a result, the presence or absence of this organism as an endogenous element of the shoulder microbiome cannot reliably be evaluated with the methods used.

PCR with 16S rRNA provides a highly sensitive method for quantifying the relative abundance of numerous bacterial species in a sample, based on the detection of rRNA sequences in 1 or more variable regions of this gene (V1, V2, V3, V4, and so on) that are unique to various bacterial taxonomic groups. This method uses PCR primers that target conserved nucleotides (occurring between variable regions) that are highly similar across most bacterial organisms and contain only a limited number of species-specific polymorphisms.[1,4] Unfortunately, the primers used in this experiment (F515 and R806, targeting the V4 region)[1] are particularly inefficient in amplifying the common skin organism *C acnes*[8] because of mismatches between the critical 3' ends of the primer sequences and the complementary annealing sites in the *C acnes* genome.[11] Of all 121 fully sequenced *C acnes* genomes currently in the Reference Sequence (RefSeq) database, fewer than 1% would be expected to amplify robustly with these primers.

This limitation as it relates specifically to *C acnes* has been previously reported[5,9] but was most comprehensively described in a recent study comparing the use of V1 to V3 vs. V4 variable regions in the analysis of human skin samples.[8] This work concluded that V4 amplification using standard primers is almost entirely unable to detect *C acnes*. As emphasized in a corresponding editorial[4] and letter,[11] alternative primers or target regions should be used in studies of the human skin microbiome because of the inability of this commonly used V4 primer set to detect *C acnes*. For this reason, subsequent studies, including the recent characterization of the microbiome of the human skin follicle,[3] have used primers targeting the V1 to V3 region. The experiments reported by Qui et al[10] include a series of negative controls (open-air collection blanks) but do not include positive controls, except for 1 PCR–restriction fragment length polymorphism assay for the single sample in which *C acnes* was detected. As such, the limitations of this specific primer pair with respect to *C acnes* identification may not have been apparent.

Although we agree with the statement of Qui et al[10] that *C acnes* is unlikely to be a normal commensal of the glenohumeral joint itself and that infections may arise from inoculation of deep structures with bacteria from more superficial tissue layers, these conclusions are not robustly supported by the results obtained. Repetition of the experiment with new or residual sample material using a primer set capable of identifying common skin commensals with less amplification bias would likely demonstrate a substantially higher rate of *C acnes* positivity at multiple anatomic levels. We further caution against the potential interpretation of positive intra-articular *C acnes* 16S PCR results as evidence that this organism is a native element of the intact shoulder microbiome, owing to the extreme sensitivity of the detection method and the well-known challenges of surgically accessing the joint space without contamination by commensal skin organisms.

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